



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 39/39, 39/145	A1	(11) International Publication Number: WO 97/00697 (43) International Publication Date: 9 January 1997 (09.01.97)												
(21) International Application Number: PCT/EP96/02690 (22) International Filing Date: 19 June 1996 (19.06.96) (30) Priority Data: <table border="0"><tr><td>9512827.8</td><td>23 June 1995 (23.06.95)</td><td>GB</td></tr><tr><td>9513443.3</td><td>1 July 1995 (01.07.95)</td><td>GB</td></tr><tr><td>9525657.4</td><td>15 December 1995 (15.12.95)</td><td>GB</td></tr><tr><td>9606032.2</td><td>22 March 1996 (22.03.96)</td><td>GB</td></tr></table> (71) Applicant (for all designated States except US): SMITHKLINE BEECHAM BIOLOGICALS S.A. [BE/BE]; Rue de l'Institut 89, B-1330 Rixensart (BE). (72) Inventors; and (75) Inventors/Applicants (for US only): PEETERMANS, Julien [BE/BE]; SmithKline Beecham Biologicals S.A., Rue de l'Institut 89, B-1330 Rixensart (BE). HAUSER, Pierre [BE/BE]; SmithKline Beecham Biologicals S.A., Rue de l'Institut 89, B-1330 Rixensart (BE). (74) Agent: DALTON, Marcus, Jonathan, William; SmithKline Beecham, Corporate Intellectual Property, SB House, Great West Road, Brentford, Middlesex TW8 9BD (GB).		9512827.8	23 June 1995 (23.06.95)	GB	9513443.3	1 July 1995 (01.07.95)	GB	9525657.4	15 December 1995 (15.12.95)	GB	9606032.2	22 March 1996 (22.03.96)	GB	(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
9512827.8	23 June 1995 (23.06.95)	GB												
9513443.3	1 July 1995 (01.07.95)	GB												
9525657.4	15 December 1995 (15.12.95)	GB												
9606032.2	22 March 1996 (22.03.96)	GB												
(54) Title: A VACCINE COMPOSITION COMPRISING A POLYSACCHARIDE CONJUGATE ANTIGEN ADSORBED ONTO ALUMINIUM PHOSPHATE (57) Abstract The invention relates to a vaccine formulation for the prevention of Haemophilus Influenzae Type B (Hib) infections and where the antigen is adsorbed onto aluminium phosphate. The invention also relates to a multivalent vaccine, that is a vaccine for the amelioration or treatment of more than one disease states. The present invention also relates to the production and use of such vaccines in medicine.														

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

A VACCINE COMPOSITION COMPRISING A POLYSACCHARIDE CONJUGATE ANTIGEN ADSORBED ONTO ALUMINIUM PHOSPHATE

The present invention relates to new vaccine formulations, comprising a conjugated polysaccharide antigen linked to a carrier protein. In particular the invention relates to a vaccine formulation for the prevention of Haemophilus Influenzae Type B (Hib) infections and where the antigen is adsorbed on to aluminium phosphate. The invention also relates to a multivalent vaccine, that is a vaccine for the amelioration or treatment of more than one disease states. The present invention also relates to the production and use of such vaccines in medicine.

Vaccines that utilise polysaccharides are known in the art. For example a vaccine for the prevention of Haemophilus influenzae b (Hib) infections are based on the capsular polysaccharide (PRP) conjugated with a carrier protein. The polysaccharide is a polymer of ribose, ribitol and phosphate. These vaccines are typically presented as plain (ie without adjuvantation) formulations. Although in one case, (Pedvax Hib produce by Merck) a diluent containing aluminium hydroxide is utilised to reconstitute the lyophilised conjugate. Typically the carrier protein is a diphtheria or tetanus toxoid or an outer membrane protein of N.meningitidis. Examples of such conjugate vaccine antigens are disclosed in US 4 365 170, US 4 673 574, EP 208 375, EP 477508 and EP 161 188.

It is desirable to administer such conjugate vaccines with other antigens or vaccines at the same time and this can involve multiple injections. Problems associated with multiple injections include a more complicated administration procedure and a large total injection volume. This is a particularly acute problem when the vaccine is intended for infants.

It has therefore been proposed to produce combination vaccines. One well known combination vaccine provides protection against Diphtheria, tetanus and B. pertussis infections. This vaccine comprises a whole cell or an acellular pertussis component which typically consists of two or three antigens - (detoxified PT, FHA and often, but not exclusively 69kDa) although in certain circumstances other B. pertussis antigens may also be present and toxoided diphtheria and tetanus

toxins. Such vaccines are often referred to as DTPw or DTPa. Other antigens would desirable be added to such a combination vaccine for the prevention of diseases like hepatitis B. or Polio.

5 It would be desirable to add polysaccharide conjugate vaccines to such a combination. However we have found that simple mixing of the components results in a reduction of antibody titres to the polysaccharide component.

The present inventors have discovered that this reduction can be inhibited if the conjugate antigen is adsorbed on to aluminium phosphate. In contrast, if the antigen is adsorbed on to aluminium hydroxide, there is a complete reduction of
10 antibody titres to the polysaccharide component.

Accordingly the present invention provides a vaccine composition comprising a polysaccharide conjugate antigen adsorbed on to aluminium phosphate. Preferably the antigen is capsular polysaccharide (PRP) from Hib conjugated with a carrier protein.

15 Preferably the carrier protein is either diphtheria or tetanus toxoid, Diphtheria Crm₁₉₇ protein or an outer membrane protein from a bacteria such as N.menigitidis.

The polysaccharide conjugate may be prepared by any known coupling technique. For example the polysaccharide can be coupled via a thioether linkage.
20 This conjugation method relies on activation of the polysaccharide with 1-cyano-4-dimethylamino pyridinium tetrafluoroborate (CDAP) to form a cyanate ester. The activated polysaccharide may thus be coupled directly or via a spacer group to an amino group on the carrier protein. Preferably, the cyanate ester is coupled with hexane diamine and the amino-derivatised polysaccharide is conjugated to the
25 carrier protein using heterologation chemistry involving the formation of the thioether linkage. Such conjugates are described in PCT published application WO93/15760 Uniformed Services University.

The conjugates can also be prepared by direct reductive amination methods as described in US 4365170 (Jennings) and US 4673574 (Anderson). Other
30 methods are described in EP-0-161-188, EP-208375 and EP-0-477508.

A further method involves the coupling of a cyanogen bromide activated polysaccharide derivatised with adipic acid hydrazide (ADH) to the protein carrier by carbodiimide condensation. Such conjugation is described in Chu C. et al *Infect Immunity*, 1983 245 256.

- 5 In a preferred embodiment of the invention the ratio of PRP polysaccharide to carrier protein is reduced from a typical 1:3 to 1:0.3 to 1:2. Such low ratio conjugates are advantageous, since even in an unadjuvanted state, they do not suffer from interference problems.

- 10 In a preferred embodiment of the invention the formulation preferably contains at least one other component selected from antigens which afford protection against one or more of the following: Hepatitis A virus (HAV), diphtheria, tetanus, pertussis, Hepatitis B and polio.

- Particular combination vaccines within the scope of the invention include a DTPa (diphtheria-tetanus-acellular pertussis) -Hib combination vaccine
15 formulation, an Hib-Hepatitis B vaccine formulation, a DTPa-Hib-Hepatitis B vaccine formulation and an IPV (inactivated polio vaccine) -DTPa-Hib-Hepatitis B vaccine formulation.

The above combinations may optionally include a component which is protective against Hepatitis A.

- 20 Suitable components for use in such vaccines are already commercially available and details may be obtained from the World Health Organisation. For example the IPV component may be the Salk inactivated polio vaccine. The Diphtheria, Tetanus and Pertussis vaccine may comprise an acellular product such as Infanrix DTPa (SmithKline Beecham Biologicals) . The component affording
25 protection against Hepatitis A is preferably the product known as 'Havrix' (SmithKline Beecham Biologicals) which is a killed attenuated vaccine derived from the HM-175 strain of HAV [see 'Inactivated Candidate Vaccines for Hepatitis A' by F.E. Andre, A Hepburn and E.D'Hondt, *Prog Med. Virol.* Vol 37, pages 72-95 (1990) and the product monograph 'Havrix' published by SmithKline Beecham
30 Biologicals (1991)]. The Hepatitis B component may comprise the 'S' antigen as in 'Engerix-B'.

Advantageously the Haemophilus Influenzae B or combination vaccine according to the invention is a paediatric vaccine.

Vaccine preparation is generally described in Vaccine Design - The Subunit and adjuvant approach Ed Powell and Newman; Penum Press. Encapsulation within liposomes is described, for example, by Fullerton, US Patent 4,235,877. Conjugation of proteins to macromolecules is disclosed, for example, by Likhite, US Patent 4,372,945 and by Armor et al., US Patent 4,474,757.

The amount of conjugate antigen in each vaccine dose is selected as an amount which induces an immunoprotective response without significant, adverse side effects in typical vaccinees. Such amount will vary depending on which specific immunogens are employed. Generally it is expected that each dose will comprise 1-1000ug of total immunogen, preferably 2-100ug, most preferably 4-40ug. An optimal amount for a particular vaccine can be ascertained by standard studies involving observation of antibody titres and other responses in subjects. Following an initial vaccination, subjects may receive one or two booster injections at about 4 weeks intervals.

In a further aspect according to the invention, there is provided a method of producing the vaccine comprising adsorbing the conjugate antigen on to aluminium phosphate. The adsorbing is preferably done at a pH of between 5 and 6, preferably at about 5.4. In an embodiment the vaccine is freeze dried after standing for more than 24 hours. Alternatively, the vaccine of the invention may be combined with other antigens in a liquid form.

The invention further provides the first medical use of such a vaccine.

In a further embodiment the invention provides a method of preventing or ameliorating Haemophilus Influenzae B infections, the method comprising the administration of a non toxic, effective amount of the vaccine of the invention.

The following examples illustrate the invention.

Example 1

Vaccine formulation comprising HiB polysaccharide conjugated on Tetanus toxoid adsorbed on to Aluminium phosphate.

5

Synthesis of Haemophilus influenzae type B capsular polysaccharide (PRP) Tetanus toxoid (TT) conjugate

1.a Cyanogen Bromide Coupling

10 The covalent binding of PRP and TT is carried out by a coupling chemistry developed at the NIH (Chu C. et al (1983), further studies on the immunogenicity of Haemophilus influenzae type b and pneumococcal type 6A polysaccharide protein conjugates. *Infect. Immunity*, 245-256). The PRP is activated under controlled conditions by cyanogen bromide and derivatised with an adipic hydrazide spacer.

15 After derivatisation, the activated polysaccharide (PRP-AH) is purified by diafiltration. The coupling of the two purified components (PRP-AH and TT) is effected by carbodiimide condensation. The conjugate is then purified by ultrafiltration and gel filtration to remove the reagent and unconjugated PRP and TT.

20

Synthesis of PRP-TT Conjugates

1.b CDAP coupling

25 30mg of native Hib PRP were dissolved in 6ml 2M NaCl. 225mcl of CDAP (1 cyano-4-dimethylamino-pyridinum tetrafluoroborate) was added to the polysaccharide solution (from a 100 mg/ml stock solution in acetonitrile). 90 seconds later, 450 mcl of 0.2 M triethylamine was added. The activation was performed at pH 10.0 during 1 minute on ice and minute at room temperature.

30 90 mg of tetanus toxoid (initial PS/protein ratio of 1/3) were added to the activated polysaccharide and the coupling reaction was performed at room

temperature for 1 hour. Then, the reaction was quenched with 3 ml of 1M glycine solution, pH 5.0 for 30 minutes at room temperature and overnight at 4°C.

The conjugate was purified by gel filtration on a sephacryl HR 500 column equilibrated in 0.2M NaCl. The carbohydrate and protein content was determined in each fraction. The conjugate was pooled and sterile filtered (membrane Minisart \oplus 0.222 μ m).

Adsorption on to aluminium phosphate

10 1.c To 0.15mg of aluminium phosphate was added 12.5 mcg of the polysaccharide conjugate of example 1(a). This was stirred for two hours the pH is adjusted to 5.1. The mixture was left to stand for one day at room temperature and the adsorbed conjugate then left for a further 9 days at 2 to 8 °C. To prepare a freeze dried product the adsorbed product is diluted in lactose (15.75mg) to give a final
15 composition of 25mcg polysaccharide/ml and 0.4mg Al/ml and the resulting composition was filled into 0.5ml vials and freeze dried.

To prepare a liquid product the adsorbed conjugate is diluted in water for injection with 150mM NaCl and 5mg/ml phenoxy ethanol to give a final composition of 20 mcg polysaccharide/ml and 0.32 mgAl/ml.

20

1.d Formulation of a Diphtheria Tetanus and Pertussis (acellular) vaccine with and without hepatitis B was done in accordance to the methods of WO 93/24148 (SmithKline Beecham Biologicals).

25 1.e Preparation of a 'low ratio' PRP-TT aluminium phosphate pre-adsorbed conjugate.

The conjugate was prepared in an analogous manner to the example of 1a, but with reduced amount of Tetanus used (30mcg, 60mcg) to give a product with

30 Polysaccharide:Protein ratio of 1:1 or 1:2. The conjugate is then adsorbed on to aluminium phosphate according to the method of example 1c. The final freeze

dried preparation contains 12.5 μ g of conjugate, 0.15mg ALPO₄, 15.75 mg lactose. This is reconstituted in 0.5ml water for injection prior to use at a pH of 0.1 +/- 0.1.

5 **Example 2: Immunogenicity of PRP-TT conjugate preadsorbed on aluminium phosphate and combined with DTPa or DTPa-HB**

The Hib conjugate of example 1a), either plain or pre-adsorbed on Al PO₄ (both vaccines were lyophilized) was mixed with DTPa or DTPa HB no more than
10 1 hour before injection and the combination was injected in baby rats (1 week of age) by the subcutaneous route at a dose corresponding to 1/20th a human dose (0.5 μ g of PRP). The rats were boosted 2 weeks and 4 weeks later and the serum was collected after each immunization to measure anti-PRP antibodies. Controls included the Hib vaccines (adsorbed or not on Al PO₄) reconstituted in
15 saline.

Groups of 10 randomized baby rats (1 week of age-OFA strain) were immunized 3 times subcutaneously at 0-14-28 days with 1/20th human dose of Hib vaccine, alone or combined with DTPa or DTPa HB (1/20th a human dose). The reconstitution of the lyophilised Hib vaccine with saline or combinations (DTPa or
20 DTPa HB) was done less than 1 hour before immunization.

The rats were bled under anesthesia at 14-28-42 and 56 days. The anti-PRP antibodies were measured by ELISA in individual sera and the titers were expressed in γ /ml using a calibrated reference. The GMT was calculated for each group and for each time point. The 95% confidence limits were calculated for the titers
25 obtained after that third immunization.

As shown in table 1, the adsorption of Hib conjugate on Al PO₄ does not modify its immunogenicity: some anti-PS were produced after the second dose and a good booster effect is shown after the third dose as seen in human babies. The mixing of Hib vaccine with DTPa or DTPa HB reduces by 3 to 8 fold the anti-PRP
30 response and, in the case of DTPa-HB, this decrease is significant. In contrast, the

pre-adsorption of the Hib vaccine on Al PO₄ restores the anti-PRP response to a level at least equivalent to that obtained with the plain vaccine.

Conclusion:

5

The Hib/aluminium phosphate formulation has thus the potential to solve the compatibility problem encountered when mixing Hib with other paediatric combinations.

Table 1

Immunogenicity in a baby rat model of PRP-TT conjugate pre adsorbed on AIP04
 5 and combined with DTPa or DTPa-HB

Vaccine	Anti-PRP titre (γ/ml) at day			
	14 (Post I)	28 (Post II)	42 (Post III)	56 (Post III 30)
None (Nacl 0.9%)	<0.05	<0.05	<0.05	<0.05
Hib-001	<0.05	0.06	<u>12.9</u> (4-37)	<u>10.9</u> (4-31)
Hib/AIP04 (Dhib-024)	<0.05	1.3	<u>11.8</u> (5-29)	<u>15.4</u> (7-35)
Hib-001 + DTPa (119)	<0.05	0.16	3.4 (0-28)	1.4 (0.1-17)
Hib/ AIP04 + DTPa (119)	<0.05	1.9	<u>20.9</u> (7-59)	<u>19.7</u> (9.42)
Hib-001 + DTPa HB (16705)	<0.05	0.14	2.8 (1-6)	3.9 (2-9)
Hib/ AIP04 + DTPa HB (16705)	<0.05	0.47	<u>11.4</u> (5-27)	<u>18.1</u> (9-38)
Hib/Al(OH ₃)	<0.05	<0.05	<0.05	<0.14

Claims:

1. A vaccine composition comprising a polysaccharide conjugate antigen adsorbed
5 onto aluminium phosphate.
2. A vaccine as claimed in claim 1, wherein the antigen is a capsular polysaccharide from *Haemophilus influenzae* B conjugate with a carrier protein.
3. A vaccine as claimed in claim 1 or 2, wherein the carrier is selected from the
group comprising Diphtheria toxoid, Diphtheria CRM₁₉₇ protein, meningococcal
10 outer membrane protein and Tetanus toxoid.
4. A vaccine as claimed in claim 1, 2 or 3 wherein the carrier protein is a tetanus toxoid.
5. A vaccine as claimed in any one of claims 1 to 4 wherein the ratio of polysaccharide to protein is from 1:0.3 to 1:2 (weight:weight).
- 15 6. A vaccine as claimed in any of claims 1 to 5 contains at least one other component to afford protection against a pathogen other than *Haemophilus influenzae* B.
7. A vaccine as claimed in any of claims 1 to 6 wherein the other component is selected from the group of antigens which afford protection against one of Hepatitis A virus, diphtheria, tetanus, pertussis, Hepatitis B and Polio.
- 20 8. A vaccine as claimed in any of claim 1 to 7 wherein the adsorbed conjugate is freeze dried.
9. A vaccine as claimed in any of claim 1 to 7 wherein the adsorbed conjugate is suspended in water for injection.
10. A kit comprising a container of a freeze dried vaccine according to claim 8 and a
25 second container with a vaccine against a second pathogen.
11. A method of producing a vaccine according to claims 1 to 6 comprising conjugating a polysaccharide antigen to a protein carrier and adsorbing said antigen onto aluminium phosphate.
12. A vaccine composition as defined in any of claims 1 to 9 for use in medicine.

13. A method of treating a patient suffering from or susceptible to *Haemophilus influenzae* b infection, comprising administering a safe and efficacious amount of a vaccine composition according to any of claims 1 to 9.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 96/02690

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K39/39 A61K39/145

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ADV. EXP. MED. BIOL., vol. 303, 1991, IMMUNOBIOLOGY OF PROTEINS AND PEPTIDES VI, ED.: M.Z. ATASSI, PLENUM PRESS, NEW YORK, 1991, pages 185-190, XP000570343 BIXLER, JR., G.S. AND S. PILLAI: "Augmentation by interleukins of the antibody response to a conjugate vaccine against Haemophilus influenza B"	1-5,8,9, 11-13
Y	see the whole document	6,7,10
Y	EP,A,0 594 950 (AMERICAN CYANAMID COMPANY) 4 May 1994	6,7,10
A	see the whole document	1-5,8,9, 11-13

	-/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

14 October 1996

Date of mailing of the international search report

15. 11. 96

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+ 31-70) 340-3016

Authorized officer

Olsen, L

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 96/02690

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>THE JOURNAL OF PEDIATRICS, vol. 112, 1988, pages 695-702, XP000604106 CLAESSON, B.A. ET AL: "Clinical and immunologic responses to the capsular polysaccharide of Haemophilus influenzae type b alone or conjugated to tetanus toxoid in 18- to 23-month-old children" see the whole document -----</p>	1-13

INTERNATIONAL SEARCH REPORT

Patent Application No
PCT/EP 96/02690

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0594950	04-05-94	AU-B- 669570	13-06-96
		AU-A- 5029093	12-05-94
		CA-A- 2109159	28-04-94
		CZ-A- 9302272	18-05-94
		FI-A- 934725	28-04-94
		HU-A- 67239	28-03-95
		JP-A- 7300427	14-11-95
		NO-A- 933856	28-04-94
		NZ-A- 250029	27-09-94
